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富含  $\beta$ -胡萝卜素的杜氏盐藻的技术开发研究

The study on the technology and development of *Dunaliella*  
which are riched in  $\beta$ -carotene

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## 摘 要

微藻在能源、医药、生物技术等有很大的应用前景, 为了实现微藻的大规模工业化生产, 微藻的培养、采收工艺、提取技术成为当前的研究重点。与其他藻种相比, 盐藻能有效的积累天然  $\beta$ -胡萝卜素, 其产品开发呈现多样化趋势, 具有较高的经济价值, 因而受到广大研究者的重视。本研究对盐藻室内生长条件及  $\beta$ -胡萝卜素积累条件进行考察; 对室外条件下盐藻生长以及  $\beta$ -胡萝卜素积累情况进行监测; 构建了一套规模化养殖工艺; 提出了两种盐藻分离工艺; 建立了植物油微波提取  $\beta$ -胡萝卜素的工艺。研究得到以下主要结论:

(1) 室内条件下 (6000Lux, 30℃), 以硝酸钠、硝酸钾作氮源, 氯化钠调节盐度培养 7-8 天, 盐藻干重可以达到 0.13 g/d, 盐藻细胞生长的最佳条件为: 氮浓度为 5.0mM、盐度为 1.5M、橘黄色光质。室内条件下 (22000Lux, 35℃),  $\beta$ -胡萝卜素积累的最佳条件为 0.5mM 的氮浓度、盐度 2.5M,  $\beta$ -胡萝卜素含量可以达到 3.64%, 经过 8 天的培养  $\beta$ -胡萝卜素产量为 3.035mg/(L·d)。

(2) 室外培养时 (从 2015.05.29-2015.06.15, 参数见附录表 3), 以硝酸钠、硝酸钾作氮源, 氯化钠调节盐度培养 18 天左右,  $\beta$ -胡萝卜素积累的最佳条件为 0.6mM 氮源, 2.5M 盐度, 积累的  $\beta$ -胡萝卜素含量为 3.14%, 产量为 2.47 mg/(L·d)。

(3) 二步法培养下藻细胞的  $\beta$ -胡萝卜素明显高于常规方法培养的藻细胞, 比常规培养的  $\beta$ -胡萝卜素高出 33.2%; 常规培养的藻细胞增长速度较快, 最高达到  $2.1 \times 10^7$  cells/mL, 比二步法培养下的藻细胞数量高出约 21.05%, 这说明了极端环境会抑制盐藻细胞的生长。

(4) 采用沙载电絮凝工艺和螺旋藻废液可以有效低耗的采收盐藻, 当通电电流为 0.5A, 沙子剂量 250mg/L, 转速 150 rpm 时的可以得到最佳采收率 97.16%, 能耗为 0.380 kWh/kg, 远低于工业中应用的离心分离的 1.67 kWh/kg, 采收过后的上清液经过氮浓度补充可以循环利用, 能够节约水资源, 降低培养成本; 采用螺旋藻废液采收工艺时, 在螺旋藻上清液与盐藻液比值为 1:4 时, 采收效果最高可达到 75.26%, 在螺旋藻上清液和培养基的混合液与盐藻的配比为 1:5 时, 沉降时间为 3h 时, 混合液的最佳采收率为 74.77%, 将螺旋藻上清液絮凝盐藻液后的上层澄清液进行培养盐藻细胞时, 配比为 2:5 的藻细胞生物量高出正常培养的

75%。

(5) 植物油萃取工艺是一种绿色安全有效的  $\beta$ -胡萝卜素萃取工艺。植物油直接萃取工艺在反应条件为温度  $50^{\circ}\text{C}$ 、油浴时间 40min、藻油比 1:5，最大采收率为 67.82%；植物油微波辅助萃取工艺在反应条件在功率 50%、微波加热时间 6min、温度  $48^{\circ}\text{C}$ 、藻油比（微藻与植物油比例）1:6 时的最高萃取率可以达到 83.60%，比直接油浴法高出 23.27%；

**关键词：**盐藻；规模化培养；采收； $\beta$ -胡萝卜素



## Abstract

Microalgae have promising applications in the energy industry, pharmaceutical industry, and biotechnology. In order to achieve large-scale industrial production of salina, microalgae cultivation, harvesting technology, extraction technology has become the focus of current research. Compared with other species of algae, *Dunaliella* which possesses a high economic value can effectively accumulate natural  $\beta$ -carotene, and its products showing a diversified trend. In this study, firstly we studied the growing conditions and  $\beta$ -carotene accumulation condition of *Dunaliella* indoor. Then, we studied corresponding conditions of growth and  $\beta$ -carotene accumulation outdoor. We build a large-scale cultivation technology. Two separation strategies were applied. Finally, a novel vegetable oil extraction technology of  $\beta$ -carotene was applied. Main conclusions were listed as the follows:

(1) Under indoor cultivation conditions: 6000Lux, 30°C, 7-8 cultivation time, using  $\text{NaNO}_3$ 、 $\text{KNO}_3$  as nitrogen source,  $\text{NaCl}$  to increase salty, the dry weight of *Dunaliella* can reach 0.13 g/d, the optimal conditions for the *Dunaliella* cells growth were under the nitrogen concentration of 5.0mM, salinity of 1.5M, and the orange light. The optimum conditions (22000Lux, 35°C) indoor for  $\beta$ -carotene accumulation were under nitrogen concentration of 0.5mM, salinity of 2.5M.  $\beta$ -carotene content was up to 3.64%, after 8 days of cultivation  $\beta$ -carotene yield was 3.035mg / (L·d).

(2) In outdoor cultivation (from 2015.05.29-2015.06.15, average illumination of  $13000\mu\text{w}/\text{cm}^2$ ), using  $\text{NaNO}_3$ 、 $\text{KNO}_3$  as nitrogen source,  $\text{NaCl}$  to increase salty, the best conditions for the  $\beta$ -carotene accumulation were 0.6mM nitrogen, 2.5M salinity. Accumulation of  $\beta$ -carotene content reached 3.14% and a yield of 2.47 mg/(L·d).

(3) Cells in normal cultivation method grew faster than the two-step cultivation method and the number of cells was approximately 21.05% higher, which suggested the extreme environment will inhibit the growth of *Dunaliella* cells. The accumulation of  $\beta$ -carotene was 33.2% higher than normal cultivation under the two-step method.

(4) Sand enhanced electric flocculation technology and used *spirulina* culture are

two effectively low harvesting technologies. When the electric current was 0.5A, speed of 150 rpm and sand dose was 250mg/L, the best recovery reached 97.16% and energy consumption was 0.380 kWh/kg, which was far below centrifugal separation (1.67 kWh/kg) applied in the industrial. Furthermore, the flocculated supernatant can be reused after supplement of nitrogen. The sand enhanced electric flocculation is a great way to save water and reduce the cost of cultivation. When the ratio of used *Spirulina* culture and *Dunaliella* broth is 1: 4, the recovery effect can reach 75.26%. Furthermore, we mixed the used *Spirulina* culture medium and fresh *Spirulina* culture medium, when the ratio of the mixture liquid and *Dunaliella* broth was 1:5, settling time was 3h, the optimum recovery was 74.77%. Using the supernatant of flocculated liquid to culture *Dunaliella* with the ratio of 2:5, the biomass of algal cells was 75% higher than normal cultivation.

(5) Microwave-assisted vegetable oil technology is a safe and effective method to extract  $\beta$ - carotene , when the reaction conditions is 50% power, 6min, 48℃, solid-liquid ratio of 1: 6, the highest recovery rate can reach 83.60 %, which is 23.27% higher than the direct vegetable oil extraction; the maximum recovery of 67.82% can be reached using vegetable oil direct extraction with the reaction conditions in 50℃, 40min, solid to liquid ratio of 1: 5.

**Key words:** *Dunaliella*; Large-scale cultivation; Harvesting;  $\beta$  -carotene;

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